CLAIMS

1. A method of preparing a hydrogel immobilised to a solid support comprising polymerising on said support a mixture of:

(i) a first comonomer which is acrylamide, methacrylamide, hydroxyethyl methacrylate or N-vinyl pyrrolidinone; and

(ii) a second comonomer which is a functionalised acrylamide or acrylate of formula (I):

$$H_2C=C(H)-C(=O)-A-B-C \quad (I);$$

or a methacrylate or methacrylamide of formula (II):

$$\text{or } H_2C=C(CH_3)-C(=O)-A-B-C- \quad (II)$$

(wherein:

A is NR or O, wherein R is hydrogen or an optionally substituted saturated hydrocarbyl group comprising 1 to 5 carbon atoms;

-B- is an optionally substituted alkylene biradical of formula $-(CH_2)_n-$ wherein n is an integer from 1 to 50; and wherein n = 2 or more, one or more optionally substituted ethylene biradicals $-CH_2CH_2-$ of said alkylene biradical may be independently replaced by ethenylene and ethynylene moieties; and wherein n=1 or more, one or more methylene biradicals $-CH_2-$ may be replaced independently with an optionally substituted mono- or polycyclic hydrocarbon biradical comprising from 4 to 50 carbon atoms, or a corresponding heteromonocyclic or heteropolycyclic biradical wherein at least 1 CH_2 or CH_2 is substituted by an oxygen sulfur or nitrogen atom or an NH group; and

C is a group for reaction with a compound to bind said compound covalently to said hydrogel) to form a polymerised product,

characterised in that said method is conducted on, and immobilises the polymerised product to, said support which is not covalently surface-modified.

2. The method as claimed in claim 1 wherein said support is a silica-based support.

3. The method as claimed in claim 2 wherein said silica-based support is fused silica.

4. The method as claimed in claim 3 wherein said silica fused silica is SPECTRASIL™.

5. The method as claimed in claim 1 wherein said support is a non silica-based support.

6. A method as claimed in any preceding claim wherein said first comonomer is acrylamide.

7. A method as claimed in any preceding claim wherein said second comonomer is an acrylamide of formula (I).

8. A method as claimed in claim 6 wherein said acrylamide of formula (I) has A = NH.

9. A method as claimed in any preceding claim wherein -B- is a C_2-C_{10} alkylene biradical.

10. The method as claimed in claim 8 wherein -B- is $-(CH_2)_5-$.

11. The method as claimed in any preceding claim wherein C is hydroxyl, thiol, amine, acid, ester or haloacetamido.

12. The method as claimed in claim 11 wherein said haloacetamido is bromoacetamido.

13. The method as claimed in any one of claims 1 to 8 wherein said acrylamide of formula (I) is *N*-(5-bromoacetamidylpentyl) acrylamide (BRAPA).

14. The method as claimed in any preceding claim wherein said second comonomer is present in an amount of ≥1 mol% relative to the total molar quantity of comonomers.

15. The method as claimed in claim 14 wherein said second comonomer is present in an amount of ≥2 mol% relative to the total molar quantity of total comonomers.

16. The method as claimed in any preceding claim wherein no polyunsaturated crosslinking agent is present during said polymerising.

17. A solid-supported hydrogel obtainable according to the method of any one of the preceding claims.

18. The solid-supported hydrogel of claim 17 wherein the thickness of the hydrogel is less than 100 nm.

19. A method of preparing a solid supported hydrogel-based molecular array, said method comprising reacting one or more molecules of interest with reactive sites present in a solid-supported hydrogel as defined in claim 17 or claim 18.

20. The method of claim 19 wherein said molecules of interest are biomolecules.

21. The method of claim 19 or claim 20 wherein said molecules of interest are polynucleotides or proteins.

22. The method of claim 21 wherein said molecules of interest are polynucleotides.

23. The method of claim 22 wherein at least a portion of each polynucleotide is single-stranded.

24. The method of claim 22 or claim 23 wherein said polynucleotides comprise from 1 to 20 spacer nucleotides.

25. The method of claim 24 wherein said polynucleotides comprise from 1 to 10 spacer nucleotides.

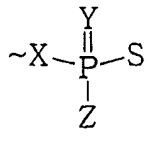
26. The method of claim 25 wherein said polynucleotides comprise 10 spacer nucleotides.

27. The method of any one of claims 24 to 26 wherein said spacer nucleotides each contain the base thymine (T).

28. The method of claim 22 or claim 23 wherein said polynucleotides are hairpin polynucleotides.

29. The method of any one of claims 19 to 28 wherein said molecules of interest contain a sulfur-containing nucleophile.

30. The method of claim 29 wherein said sulfur-containing nucleophile is a moiety of the formula (III):



(III)

(wherein ~ denotes the bond or linker connecting the sulfur-based nucleophile to the remainder of the polynucleotide; X represents an oxygen atom, a sulfur atom or a group NR, in

which R is hydrogen or an optionally substituted C_{1-10} alkyl; Y represents an oxygen or a sulfur atom; and Z represents an oxygen atom, a sulfur atom or an optionally substituted C_{1-10} alkyl group).

31. The method of claim 30 wherein X is oxygen or sulfur.

32. The method of claim 30 or claim 31 wherein Y is oxygen.

33. The method of any one of claims 30 to 32 wherein Z is an oxygen or sulfur atom or a methyl group.

34. The method of any one of claims 30 to 33 wherein said moiety is thiophosphate.

35. The method of any one of claims 29 to 34 wherein said sulfur-containing nucleophile is connected to the molecule of interest by a linker group and wherein said molecule of interest is a polynucleotide.

36. A method of preparing a solid supported hydrogel-based molecular array which is a clustered array of molecules of interest, the method comprising:

(i) reacting polynucleotide molecules with reactive sites present in a solid-supported hydrogel according to the method of any one of claims 22 to 27, wherein said polynucleotide molecules are first and second oligonucleotide primers capable of hybridising to a template to be amplified;

(ii) contacting the first oligonucleotide primers attached to the solid-supported hydrogel in step (i) with one or more templates to be amplified under conditions which permit hybridisation of the templates to the oligonucleotide

primers, each template comprising at the 3' end a sequence capable of hybridising to the first oligonucleotide primer and at the 5' end a sequence the complement of which is capable of hybridising to a second oligonucleotide primer; and

(iii) performing one or more nucleic acid amplification reactions using the first and second oligonucleotide primers and the template(s), thereby generating a clustered array of molecules of interest.

37. A method of modifying a molecular array, which molecular array comprises a plurality of molecules of interest immobilised to a surface of a support, said method comprising the step of applying to the array polyelectrolyte or neutral polymers.

38. The method of claim 37 wherein said molecules of interest are as defined in any one of claims 20 to 36.

39. The method of claim 37 or claim 38 wherein the support is comprised of a member selected from the group comprising silica-based substrates, hydrogels and polyelectrolyte multilayers.

40. The method of claim 39 wherein the molecules of interest are attached directly or through a linking moiety to a silica-based support.

41. The method of claim 39 wherein the hydrogel is a polyacrylamide hydrogel.

42. The method of claim 39 wherein the polyelectrolyte multilayer comprises one or more layers of each of polyallylamine and polyacrylic acid wherein the surface to

which the biomolecules are attached comprises polyacrylic acid.

43. The method of claim 39 wherein the hydrogel is obtainable by a method comprising polymerising on a solid support a mixture of:

(i) a first comonomer which is acrylamide, methacrylamide, hydroxyethyl methacrylate or N-vinyl pyrrolidinone; and

(ii) a second comonomer which is a functionalised acrylamide or acrylate of formula (I):

$$H_2C=C(H)-C(=O)-A-B-C \quad (I);$$

or a methacrylate or methacrylamide of formula (II):

$$\text{or } H_2C=C(CH_3)-C(=O)-A-B-C- \quad (II)$$

(wherein:

A is NR or O, wherein R is hydrogen or an optionally substituted saturated hydrocarbyl group comprising 1 to 5 carbon atoms;

-B- is an optionally substituted alkylene biradical of formula $-(CH_2)_n-$ wherein n is an integer from 1 to 50; and wherein n = 2 or more, one or more optionally substituted ethylene biradicals $-CH_2CH_2-$ of said alkylene biradical may be independently replaced by ethenylene and ethynylene moieties; and wherein n=1 or more, one or more methylene biradicals $-CH_2-$ may be replaced independently with an optionally substituted mono- or polycyclic hydrocarbon biradical comprising from 4 to 50 carbon atoms, or a corresponding heteromonocyclic or heteropolycyclic biradical wherein at least 1 CH_2 or CH_2 is substituted by an oxygen sulfur or nitrogen atom or an NH group; and

C is a group for reaction with a compound to bind said compound covalently to said hydrogel) to form a polymerised

product wherein said polymerising is conducted on, and immobilises the polymerised product to, said solid support.

44. The method of claim 43 wherein the hydrogel is obtainable by a method as defined in any one of claims 1 to 16.

45. The method of any one of claims 37 to 44 wherein the polyelectrolyte applied is polyacrylic acid.

46. The method of any one of claims 37 to 45 wherein polyallylamine is applied to the array followed by polyacrylic acid.

47. The method of any one of claims 37 to 44 wherein the neutral polymer is polyethylene glycol.

48. The method of any one of claims 37 to 47 wherein the method comprises modifying a microarray or a single molecule array.

49. The method of claim 48 wherein the method comprises modifying a single molecule array.

50. The method of claim 48 wherein the method comprises modifying a clustered microarray.

51. A molecular array obtainable according to the method of any one of claims 19 to 50.

52. The molecular array of claim 51 which is a single molecule array.

53. The molecular array of claim 51 which is a clustered microarray.

54. Use of a molecular array as defined in any one of claims 51 to 53 in any method requiring interrogation of the immobilised molecules of interest or molecules bound thereto.

55. Use as claimed in claim 54 wherein said immobilised molecules of interest are polynucleotides and the method is a sequencing reaction for determining the sequence of the whole or a portion of said polynucleotides.

56. Use as claimed in claim 55 wherein the sequencing reaction comprising incorporating one or more nucleotides into a strand of nucleic acid complementary to the polynucleotides to be sequenced and determining the identity of the base present in one or more of the incorporated nucleotide(s).

57. Use of a solid-supported hydrogel array in a single-molecule array application wherein said arrays are obtainable by a method of comprising the steps of

(1) preparing a hydrogel immobilised to a solid support comprising polymerising on said support a mixture of:

(i) a first comonomer which is acrylamide, methacrylamide, hydroxyethyl methacrylate or N-vinyl pyrrolidinone; and

(ii) a second comonomer which is a functionalised acrylamide or acrylate of formula (I):

$$H_2C=C(H)-C(=O)-A-B-C \quad (I);$$

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A is NR or O, wherein R is hydrogen or an optionally substituted saturated hydrocarbyl group comprising 1 to 5 carbon atoms;

-B- is an optionally substituted alkylene biradical of formula $-(CH_2)_n-$ wherein n is an integer from 1 to 50; and wherein n = 2 or more, one or more optionally substituted ethylene biradicals $-CH_2CH_2-$ of said alkylene biradical may be independently replaced by ethenylene and ethynylene moieties; and wherein n=1 or more, one or more methylene biradicals $-CH_2-$ may be replaced independently with an optionally substituted mono- or polycyclic hydrocarbon biradical comprising from 4 to 50 carbon atoms, or a corresponding heteromonocyclic or heteropolycyclic biradical wherein at least 1 CH_2 or CH_2 is substituted by an oxygen sulfur or nitrogen atom or an NH group; and

C is a group for reaction with a compound to bind said compound covalently to said hydrogel) to form a polymerised product, and

(2) attaching one or more molecules of interest to reactive sites present in the hydrogel produced in step (1).

58. The use as claimed in claim 57 wherein said support is a silica-based support.

59. The use as claimed in claim 57 or 58 wherein prior to the polymerising in step (1) the support is reacted with a silane binder.

60. The use as claimed in claim 59 wherein the silane binder is 3-methacryloxypropyltrimethoxysilane.